

signal for undergoing rapid fusion with the PM within seconds. The excess membrane added to the PM is retrieved via compensatory endocytosis on a longer time-scale (minutes). We have been using endocrine BON cells, a cell line derived from a human carcinoma tumor, which are capable of calcium-regulated secretion. Secretion is stimulated, almost instantly during tether extrusion, by UV-uncaging of a photolabile calcium chelator loaded into the cytosol. Thus, a significant transient membrane addition to the PM can be turned on at will and the consequences for extrusion dynamics can be studied.

We observe mainly two types of response upon stimulation: a sudden jump in tether length L of $\sim 10\ \mu\text{m}$ followed by an increased extrusion velocity, or a sudden increase of slope. Both responses are consistent with exocytotic addition of area to the PM. However, only a fraction of such responses are well-correlated with UV-uncaging of intracellular calcium. This suggests exocytosis, or other mechanisms of membrane addition to the PM may independently be triggered by other means, possibly by tether pulling itself.

Membrane Fusion

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Molecular Mechanisms of Vesicle Fusion

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We have performed large scale molecular dynamics simulations using coarse-grained lipid models to study the fusion of vesicles. Our earlier work showed that lipid tail splay played an important role in the first lipids to cross from one vesicle to another. In this work we study the effect of different lipid types on the molecular mechanism of the initial fusion events. Lipid types studied include phosphatidylcholine and phosphatidylethanolamine lipids. In order to examine the importance of lipid splay, lipids with symmetric and asymmetric tail lengths have been studied. We calculate the free energy barrier to fusion by using the umbrella sampling method to determine the potential of mean force as a function of the distance between the center of mass of the two vesicles. We will discuss the comparison of the different curves for the different lipid types and the role of molecular mechanism(s) in vesicle fusion. We will also discuss how the interactions between lipid molecules influence fusion.

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Imaging Single Virus Fusion Reveals the HIV-1 Entry Pathway

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Viruses whose fusion proteins are activated by interactions with cellular receptors at neutral pH, including HIV-1, are assumed to fuse directly with the plasma membrane. However, direct virus fusion at the cell surface has not been explicitly demonstrated. To differentiate between HIV-1 fusion with the plasma membrane (PM) and with endosomal membrane (EM), we performed time-resolved single virus imaging. Pseudoviruses bearing HIV-1 Env glycoprotein were generated and co-labeled with a content marker (Gag-GFP) and a red lipophilic membrane dye. Upon virus maturation, the Gag-GFP is cleaved by viral protease yielding a smaller GFP-tagged fragment that is readily released from virions permeabilized with saponin. This marker thus provided a convenient means to detect small pore formation during virus-cell fusion. Double-labeled viruses were adhered in the cold to HeLa-derived cells expressing CD4 and coreceptors. Fusion was triggered by shifting to 37°C and monitored by laser scanning confocal microscopy. Imaging of single HIV-cell fusion revealed the occurrence of both PM and EM fusion events. Fluorescent viruses undergoing PM fusion transferred their lipid marker (hemifusion), but not content marker (full fusion). By contrast, full fusion with an endosome was consistently observed. These EM fusion events were manifested in disappearance of a content marker, but not the lipid dye due to its limited dilution in an endosomal membrane. These results demonstrate that, contrary to a common perception, HIV-1 enters HeLa-derived target cells by receptor/coreceptor-mediated endocytosis followed by fusion with endosomes and delivery of viral nucleocapsid into the cytosol.

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The Gaussian Curvature Elastic Energy of Membrane Fusion Intermediates, and a Possible Mode of Action of Fusion-Mediating Proteins

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Calculating the Gaussian curvature elastic energy of fusion intermediates requires knowledge of the Gaussian curvature elastic modulus, κ . κ can be measured for lipids that form Q_{II} phases. It is shown that one can estimate κ for non- Q_{II} phase lipids by studying phase behavior of lipid mixtures. κ is between -5 and $-10\ k_B T$ for biological lipid compositions. The Gaussian curvature elastic energy of fusion intermediates is positive and \geq the total bending energy cal-

culated previously: it increases the total energy of fusion intermediates by $100\ k_B T$ or more. This large contribution makes the predicted intermediate energies compatible with observed lipid phase behavior in excess water. An order-of-magnitude fusion rate equation is used to show that a current theory now predicts stalk energies that are slightly too large, by about $30\ k_B T$, to rationalize the observed rates of stalk-mediated processes in pure lipids. Despite this discrepancy, when the effect of κ is included, current models of fusion intermediate energy can make semi-quantitative predictions about how proteins mediate biomembrane fusion. Fusion-mediating proteins must lower the stalk energy by several tens of $k_B T$ relative to lipidic stalks. One way proteins could do this is by altering the elastic constants of the patches of monolayer that fuse, by inserting peptides. Preliminary data using fusion peptides and membrane-spanning peptides is compatible with this role for peptides. The energies of stalks, fusion pores, rhombohedral (R) phase and Q_{II} phase relative to L_α phase all depend on approximately the same assembly of monolayer elastic constants; $(2\kappa_m\delta J_s - \kappa)$. Thus the influence of peptides on stalk and fusion pore energy can be studied by measuring the effects of peptides on R and Q_{II} phase stability (Siegel, Biophys. J.; Dec. 2008).

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Determination of Free Energy Barriers to Initial Fusion of Vesicles

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The fusion of two vesicles is studied by means of molecular dynamics simulations using coarse-grained lipid models. We use the umbrella sampling method to determine the potential of mean force as a function of the distance between the center of mass of two vesicles. The free energy barrier to the initial fusion step between two vesicles is determined from the peak in the PMF curve calculated at a center of mass distance just prior to the initial fusion step. The two CG lipid models used were the original model by Marrink, de Vries, and Mark (J. Phys. Chem. B 2004, 108,750) and its recently improved and extended version, the MARTINI model. We find that the free energy barrier for the initial fusion event varies by more than an order of magnitude between the two models. The source of the difference is found in the greater repulsive character of the interaction between the hydrophobic tail particles and the charged head-group particles in the MARTINI model compared to the older model. This interaction results in the lipid tails being confined to the hydrophobic region of the vesicle to a greater extent and the splay of the lipid tails being limited. These factors reduce the probability of triggering an initial fusion event.

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Insights Into The Energetics Of Neuronal SNARE Complex Formation

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Eukaryotic cells transport material between intracellular compartments by vesicles that bud from a donor and afterwards fuse with a target organelle. Best-studied is the molecular machinery that drives the Ca^{2+} -dependent release of neurotransmitters from synaptic vesicles. Key players in the exocytotic fusion are three proteins synaptobrevin 2, syntaxin 1a, and SNAP-25. Synaptobrevin resides in synaptic vesicles, whereas syntaxin and SNAP-25 are anchored in the plasma membrane. They belong to the so-called SNARE protein family, which members are involved in all other vesicle fusion steps. SNARE proteins are tail-anchored membrane proteins that assemble into a stable membrane-bridging complex. As SNARE assembly is accompanied by extensive structural rearrangements from mostly unstructured monomers into a tightly packed parallel four-helix bundle, it is thought that zipper-like formation of the SNARE bundle between opposing membranes provides the energy that drives fusion. It is unclear, however, whether the assembly energy indeed suffices for membrane merger. Unfortunately, a marked hysteresis in the folding and unfolding transition of the SNARE complex prevents the direct determination of the free energy of assembly. We have now investigated the assembly process by isothermal titration calorimetry. We found that the structural changes upon assembly are reflected in extremely large favorable enthalpy changes, counterbalanced by a large positive entropy change. Moreover, as SNARE complex is essentially irreversible, we made use of the fact that assembly in vitro proceeds in discrete steps. This allowed us to assess the energetics of each assembly step individually.

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Clustering of Syntaxin-1A in Model Membranes is Modulated by Phosphatidylinositol-4,5-bisphosphate and Cholesterol

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Syntaxin-1A is part of the SNARE complex that forms in membrane fusion during neuronal exocytosis of synaptic vesicles. Together with SNAP-25, the single-span transmembrane protein syntaxin-1A forms the receptor complex on